Journal of Ethnopharmacology 155 (2014) 1219–1226

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Research paper

Enzyme inhibition, antioxidant and immunomodulatory activities, and brine shrimp toxicity of extracts from the root bark, stem bark and leaves of *Terminalia macroptera*

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ARTICLE INFO

Article history:
Received 19 February 2014
Received in revised form
5 June 2014
Accepted 2 July 2014
Available online 10 July 2014

Keywords:
Terminalia macroptera
α-Glucosidase
15-Lipoxygenase
Xanthine oxidase
DPPH
Immunomodulation

ABSTRACT

Ethnopharmacological relevance: The root bark, stem bark and leaves of *Terminalia macroptera* have been traditionally used against a variety of ailments such as wounds, hepatitis, malaria, fever, cough, and diarrhea as well as tuberculosis and skin diseases in African folk medicine. Boiling water extracts of *Terminalia macroptera*, administered orally, are the most common preparations of this plant used by the traditional healers in Mali. This study aimed to investigate the inhibition of the activities of α-glucosidase, 15-lipoxygenase and xanthine oxidase, DPPH scavenging activity, complement fixation activity and brine shrimp toxicity of different extracts obtained by boiling water extraction (BWE) and by ASE (accelerated solvent extraction) with ethanol, ethanol–water and water as extractants from different plant parts of *Terminalia macroptera*.

Materials and methods: 27 different crude extracts were obtained by BWE and ASE from root bark, stem bark and leaves of *Terminalia macroptera*. The total phenolic and carbohydrate contents, enzyme inhibition activities (α-glucosidase, 15-lipoxygenase and xanthine oxidase), DPPH scavenging activity, complement fixation activity and brine shrimp toxicity of these extracts were evaluated. Principal component analysis (PCA) was applied for total biological activities evaluation.

Results: Several of the extracts from root bark, stem bark and leaves of *Terminalia macroptera* obtained by BWE and ASE showed potent enzyme inhibition activities, radical-scavenging properties and complement fixation activities. None of the extracts are toxic against brine shrimp larvae in the test concentration. Based on the results from PCA, the ASE ethanol extracts of root bark and stem bark and the low molecular weight fraction of the 50% ethanol–water extract of leaves showed the highest total biological activities. The boiling water extracts were less active, but the bark extracts showed activity as α-glucosidase inhibitors and radical scavengers, the leaf extract being less active.

Conclusion: The observed enzyme inhibition activities, radical scavenging properties and complement fixation activities may explain some of the traditional uses of this medicinal tree, such as in wound healing and against diabetes.

1. Introduction

Terminalia macroptera Guill. & Perr. (Combretaceae) is a tree that mostly grows in West Africa, and occasionally as far as Sudan and Uganda. The root bark, stem bark and leaves of the tree are used frequently in traditional African folk medicine. *Terminalia macroptera* is used against a variety of ailments such as wounds, hepatitis, malaria, fever, cough, diarrhea as well as tuberculosis and skin diseases (Diallo et al., 2002; Sanon et al., 2003; Pham et al., 2011a). The stem bark and leaves are most commonly used against sores and wounds, pain, cough, tuberculosis and hepatitis (Pham et al., 2011a). The roots are used against hepatitis, gonorrhea and various infectious diseases, including *Helicobacter pylori*-associated diseases (Silva et al., 1996, 1997, 2000, 2012; Pham et al., 2011a). Flavonoids (Nongonierma et al., 1987, 1988, 1990), triterpenoids (Conrad et al., 1998, 2001a), ellagitannins (Silva et al., 2000; Conrad et al., 2001b;

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http://dx.doi.org/10.1016/j.jep.2014.07.004
0378-8741/

Pham et al., 2011b) and other phenolics (Conrad et al., 2001a) have been identified from different parts of *Terminalia macroptera*.

A survey of the use of *Terminalia macroptera* by traditional healers in Mali indicated that diabetes was one of the illnesses mentioned (Pham et al., 2011a). Diabetes is a common metabolic disease characterized by abnormally high plasma glucose levels. α-Glucosidase is a key enzyme which catalyzes the final step in the digestive process of carbohydrates in mammals (Anam et al., 2009). An α-glucosidase inhibitor can reduce postprandial plasma glucose levels and suppresses postprandial hyperglycemia (Gao et al., 2008; Adisakwattana et al., 2011).

15-Lipoxygenase (15-LO) is an enzyme present in multiple systems that reacts with polyunsaturated fatty acids, producing active lipid metabolites which are involved in many diseases such as cancer, atherosclerosis and diabetes (Schneider and Bucar, 2005; Dobrian et al., 2011). Xanthine oxidase (XO) is a prooxidative enzyme that generates reactive oxygen species (ROS) in vascular cells (Pacher et al., 2006). Inhibition of 15-LO and XO may reduce the production of ROS which cause oxidative stress. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a frequently used method to estimate antioxidant capacities in extracts and naturally occurring compounds.

The complement system is an important part of the human immune defense, acting as a primary defense against bacterial invasions and viral infections. Complement fixating activity of polysaccharides from plants has previously been shown to be indicative of effects on the immune system (Michaelsen et al., 2000; Inngjerdingen et al., 2013).

Brine shrimp (*Artemia salina*) larvae have been used for over 30 years in toxicological studies (Meyer et al., 1982). To our knowledge, only the toxicity of crude extracts (dichloromethane, methanol, ethanol and butanol) and some isolated compounds from leaves of *Terminalia macroptera* has been reported previously (Pham et al. 2014); it was thus of interest to investigate the toxicity of other extracts from this tree.

Water decoctions of *Terminalia macroptera*, administered orally, are the most common preparations used by the traditional healers in Mali (Pham et al., 2011a). Thus, the boiling water extracts (BWE) would be expected to contain bioactive compounds present in the plant material. Traditionally in laboratory studies, low molecular weight and lipophilic compounds are extracted from plant material by the Soxhlet extraction method. Accelerated solvent extraction (ASE) for these types of compounds was first described by Ezzell et al. (1995), and it has grown steadily in use since that time (Richter and Raynie, 2012). Under elevated temperature and pressure, an extraction solvent can be used above its boiling point but still remain in the liquid state, thus improving the kinetics of the extraction process and leading to a significant decrease in solvent consumption and extraction time (Wang et al., 2010). ASE has been applied for extracting components from environment samples, biological materials, plant materials, dietary compounds, feeds, and food. It was thus of interest to compare the activities of bioactive extracts from medicinal plant by ASE and BWE.

Using leaves as a source of traditional medicines will reduce serious damage to the tree compared to the use of root and stem bark. Therefore, in this study, ASE and BWE were employed to obtain different extracts from leaf, root bark and stem bark from *Terminalia macroptera*. The aims of this study are comparing the enzyme inhibition activities, antioxidant activities, immunomodulating activities and toxicities of extracts by ASE with the conventional method (BWE), as well as to elucidate whether the activities differ among different plant parts. While some extracts and compounds from the leaves of this plant have been investigated for some biological activities (Pham et al., 2014), the activities of bark extracts, the activities of different extracts, and statistical comparison between different extracts have to our knowledge not been reported previously.

2. Materials and methods

2.1. Plant material

Leaves, root bark and stem bark of *Terminalia macroptera* were collected in Mali and identified by the Department of Traditional Medicine (DMT), Bamako, Mali. A voucher specimen is deposited at the herbarium of DMT (Voucher no. 2468/DMT). The plant material was washed, cut into small pieces, dried and pulverized to a powder with a knife mill (Brabender, Duisburg, Germany).

2.2. Extraction

2.2.1. BWE, boiling water extraction

BWE was carried out in the way traditional healers in Mali make water decoctions. Powdered root bark, stem bark or leaves (200 g of each) were placed in a pot, and extracted twice with boiling distilled water (2 L followed by 1 L) for 30 min each time. The extracts were centrifuged and filtered through Whatman No. 1 filter paper. Some of the filtrates were lyophilized and kept for further studies. These lyophilized samples were named TRB, TSB and TL for root bark, stem bark and leaves, respectively. The rest of the filtrates were subjected to ultrafiltration (cut off 5000 Da), and the low molecular weight (LMW) fractions and high molecular weight (HMW) fractions were lyophilized. TRBL, TSBL and TLL were LMW fractions from water extracts of root bark, stem bark and leaves (Fig. 1). These fractions were assayed for total phenolic content (TPC), total carbohydrate content (TCC), inhibition of enzymes (α-glucosidase, 15-lipoxygenase, xanthine oxidase), DPPH scavenging, complement fixation assay and brine shrimp toxicity assay. For method descriptions, see below.

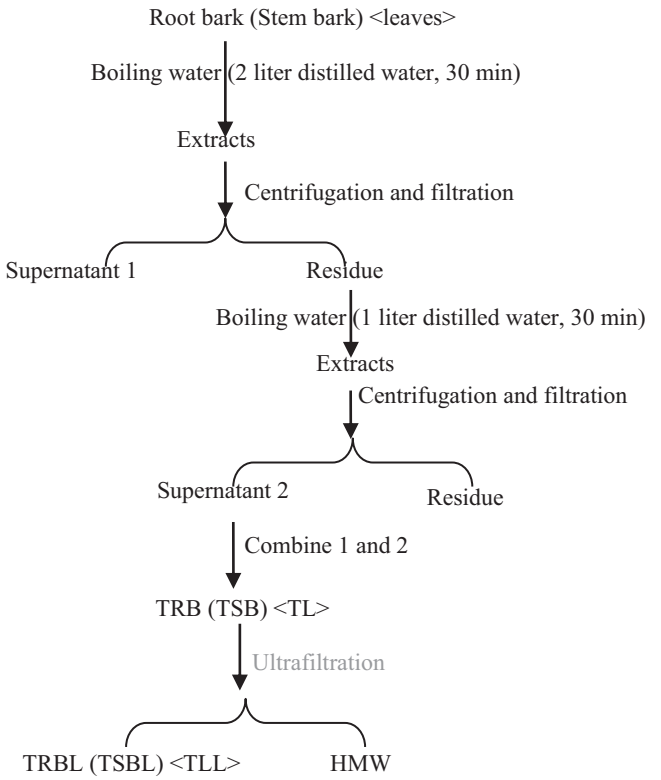

Fig. 1. Extraction scheme of crude extracts from root bark, stem bark and leaves of *Terminalia macroptera* by boiling water extraction (BWE).

2.2.2. ASE, accelerated solvent extraction

ASE was performed on a Dionex ASE350 Accelerated Solvent Extractor (Dionex, Sunnyvale, CA, USA). Powdered root bark, stem bark or leaves (200 g of each) were mixed with 50 g of diatomaceous earth, and then packed in eight 100 mL stainless steel cells. The extractions were performed at 1500 psi, with 5 min heating, 5 min static time, and a 60 s purge for a total of three cycles. The cells were extracted with 96% ethanol at 70 °C, followed by 50% ethanol–water at 70 °C, distilled water at 50 °C and 100 °C, twice of each solvent. The 96% ethanol extracts and part of 50% ethanol–water extracts and water extracts were dried and kept for further studies. These extracts were named 96TRB, 50ETRB, 50WTRB, and 100WTRB for root bark, 96TSB, 50ETSB, 50WTSB, and 100WTSB for stem bark, and 96TL, 50ETL, 50WTL, and 100WTL, for leaves. The rest of 50% ethanol–water extracts and water extracts were subjected to ultrafiltration (cut off 5000 Da), and lyophilization as above, and kept for further studies. 50ETRBL, 50WTRBL, and 100WTRBL were LMW fractions from root bark, 50ETSBL, 50WTSBL, and 100WTSBL from stem bark and 50ETLL, 50WTLL, and 100WTLL from leaves (Fig. 2). These fractions were subjected to TPC and TCC determination, DPPH scavenging, inhibition of enzymes, complement fixation assay, and brine shrimp toxicity assay.

2.3. Bioassays – general comments

All assays were done by well established methods. DMSO, which was used as solvent for our samples in most of the assays, is a good solvent and is itself inactive in our assays at the concentrations used. Sample concentrations were chosen to get activities both below and above 50% activity, unless the samples had lower activity at the highest concentration tested.

2.4. Total phenolic content

The total amounts of phenolic compounds (TPC) in the crude extracts were quantitatively determined using the Folin–Ciocalteu assay (Singleton and Rossi, 1965). A standard curve was plotted using gallic acid. TPC values were expressed as gallic acid equivalent (g GAE)/100 g plant extracts.

2.5. Total carbohydrate content

The total carbohydrate contents (TCC) in the crude extracts were determined using the phenol–sulfuric acid method (Dubois et al., 1956). A standard curve was plotted using glucose. TCC values were expressed as glucose equivalent (g Glc)/100 g plant extracts.

2.6. Inhibition of α-glucosidase

Test substances were dissolved in DMSO (> 99.5%, Sigma-Aldrich), and the assay was carried out as reported previously (Matsui et al., 1996; Bräunlich et al., 2013). α-Glucosidase from *Saccharomyces cerevisiae* and 4-nitrophenyl α-D-glucopyranoside (PNP-G) were purchased from Sigma-Aldrich (St Louis, MO, USA). Acarbose, a well-known α-glucosidase inhibitor, was employed as positive control (Calder and Geddes, 1989), and DMSO as negative control. Final DMSO concentration was 1.00%.

2.7. Inhibition of 15-lipoxygenase (15-LO)

Test substances were dissolved in DMSO, and the assay was carried out as reported previously (Wangensteen et al., 2004). Briefly, linoleic acid was peroxidized with 15-LO in the absence and presence of test substance, and the absorption increase at 234 nm was measured. Quercetin (Sigma-Aldrich) was used as positive control (Lyckander and Malterud, 1992), and DMSO as negative control. Final DMSO concentration was 1.67%.

2.8. Inhibition of xanthine oxidase (XO)

Test substances were dissolved in DMSO, and the assay was carried out as reported previously (Noro et al., 1983; Bräunlich et al., 2013), measuring XO-induced oxidation of hypoxanthine as absorption increase at 290 nm. Quercetin (Sigma-Aldrich) was

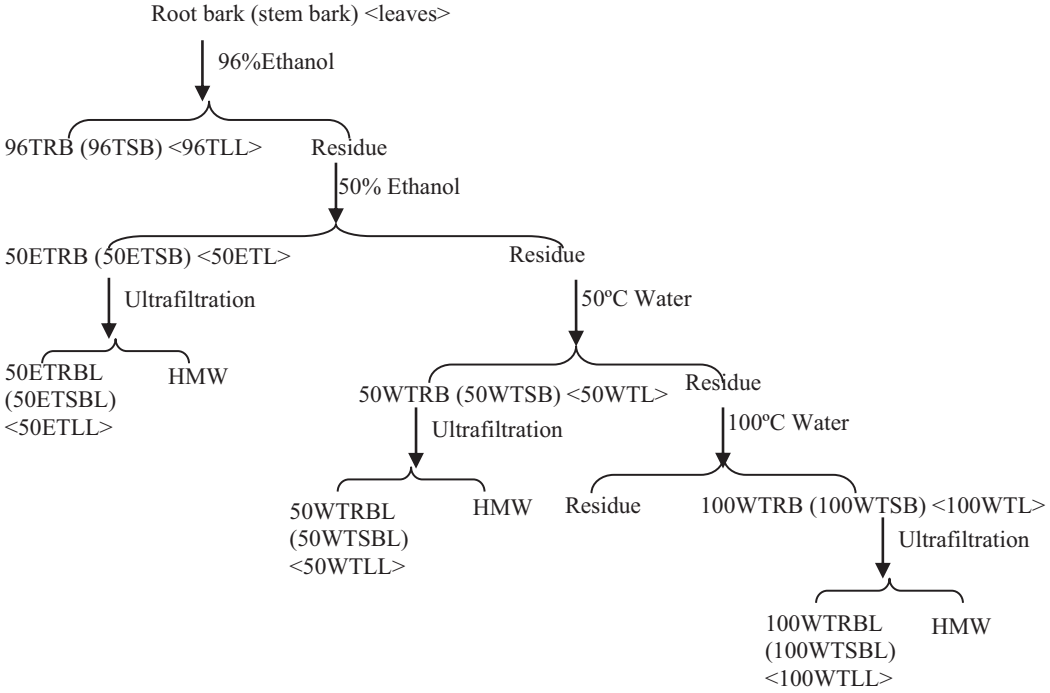

Fig. 2. Extraction scheme of crude extracts from root bark, stem bark and leaves of *Terminalia macroptera* by accelerated solvent extraction (ASE).

used as positive control (Pham et al., 2011b), and DMSO as negative control. Final DMSO concentration was 1.67%.

2.9. DPPH scavenging

Test substances were dissolved in DMSO, and the assay was carried out as reported previously (Wangensteen et al., 2004) by measuring the decrease in absorbance at 517 nm over a 5-min period. Quercetin (Sigma-Aldrich) was used as positive control (Bräunlich et al., 2013), and DMSO as negative control. Final DMSO concentration was 1.67%.

2.10. Complement fixation assay

The complement fixation test is based on inhibition of hemolysis of antibody sensitized sheep red blood cells (SRBC) by human sera as described by Michaelsen et al. (2000) (Method A). BPII, a highly active pectic polysaccharide from the aerial parts of *Biophytum petersianum* Klotzsch (Grønhaug et al., 2011), was used as a positive control.

2.11. Brine shrimp toxicity assay

Test substances were dissolved in DMSO, and the assay was carried out as reported previously (Meyer et al., 1982). Podophyllotoxin was used as a positive control, and DMSO as negative control. Final DMSO concentration was 1.00%.

2.12. Statistical analysis

All samples were analyzed in triplicates and the results are shown as mean ± standard deviation (S.D.). Analysis of correlations was determined by bivariate correlations test using IBM SPSS Statistics V21.

3. Results and discussion

3.1. Extracts from root bark

The present study shows high levels of total phenolic constituents (TPC) in the extracts of root bark of *Terminalia macroptera* (Table 1). The TPC of different extracts from root bark ranged from 2.7 to 34.9 g GAE/100 g extract. The 96TRB showed the highest TPC, while 100TRBL showed the lowest. The total phenolic content of original crude extracts TRB obtained by extraction with boiling water was more than twice that of the LMW fraction TRBL, which shows that a considerable amount of phenolic compounds was present in HMW fraction. For the ASE, 96% ethanol extracts contain the highest amount of TPC, followed by 50% ethanol–water, 50 °C and 100 °C water extracts. The TPC present in 96TRB was higher than that of TRB, which indicates that ethanol extraction with ASE is a more efficient method for obtaining phenolic compounds than BWE.

The TCC (total carbohydrate content) of extracts from root bark ranged from 12.8 to 82.8 g glucose equivalents/100 g extracts. The results indicate that the original crude extracts contain more carbohydrates than LMW fractions, both by BWE and ASE, analogously to what was found for TPC. The TCC in 100TRB was higher than that in TRB, which indicated that ASE is more efficient to obtain carbohydrate compounds than BWE. Both in crude extracts from BWE and ASE, the original fractions contain higher amount of TPC and TCC than their LMW fractions, which shows that a considerable amount of TPC and TCC was present in the HMW fractions. The phenolic compounds present in HMW fractions are probably mainly due to the phenolic compounds that are present as cross-links between polysaccharides in cell wall (Bunzel et al., 2004), and/or being linked as ester to Ara and Gal in pectins (Levigne et al., 2004).

All extracts (except 100WTRBL) obtained from root bark showed very strong α-glucosidase inhibition activity compared to the positive control acarbose. The original crude extracts showed lower IC_{50} values (higher activities) than LMW fractions, both from BWE and ASE. For the ASE extracts, the 96TRB and 50ETRB showed the highest α-glucosidase inhibition activities, followed by 50 °C and 100 °C water extracts. The α-glucosidase inhibition activity of 96TRB (IC_{50} 0.5 ± 0.1 μg/mL) was higher than that of TRB (1.6 ± 0.1 μg/mL), indicating that ethanol extraction with ASE is a more efficient method to obtain α-glucosidase inhibitors than BWE.

96TRB obtained by ASE exhibited the highest 15-LO (IC_{50} 53.7 ± 2.9 μg/mL) and XO (IC_{50} 26.0 ± 5.2 μg/mL) inhibitory activities, similar to the results from α-glucosidase inhibition assay. The 50 °C and 100 °C water extracts were inactive as 15-LO and XO inhibitors; thus, most of the inhibitors were extracted with 96% and 50% ethanol.

All extracts (except 100WTRBL) obtained from root bark showed very strong DPPH radical scavenging activity (IC_{50} < 40 μg/mL). The original crude extracts showed higher activities than the LMW fractions, both from BWE and ASE, showing that some of the radical scavenging activity lies in the high molecular weight part of the extracts. For the ASE, the 96TRB and 50ETRB

Table 1
Total phenolic content, total carbohydrate content, inhibition of α-glucosidase, 15-LO, and XO, DPPH radical scavenging and complement fixation activity of extracts from root bark of *Terminalia macroptera*.

	TPC GAE ± S.D.[a]	TCC Glc ± S.D.[b]	α-Glucosidase IC_{50} ± S.D.[c]	15-LO IC_{50} ± S.D.[c]	XO IC_{50} ± S.D.[c]	DPPH IC_{50} ± S.D.[c]	Complement fixation IC_{50} ± S.D.[c]
TRB	30.7 ± 0.8	38.4 ± 1.7	1.6 ± 0.1	58.8 ± 2.3	145 ± 5	6.2 ± 0.3	43.6 ± 5.2
TRBL	13.0 ± 0.2	27.1 ± 4.1	5.3 ± 0.3	118 ± 6	> 250	10.9 ± 0.4	30.1 ± 2.8
96TRB	34.9 ± 1.1	36.9 ± 0.8	0.5 ± 0.1	53.7 ± 2.9	26.0 ± 5.2	5.3 ± 0.1	166 ± 2
50ETRB	32.5 ± 0.6	32.9 ± 3	0.5 ± 0.1	70.0 ± 2.2	138 ± 4	5.1 ± 0.7	70.1 ± 5.2
50ETRBL	16.2 ± 0.1	27.3 ± 4.6	3.3 ± 0.2	106 ± 15	> 250	11.8 ± 0.6	61.2 ± 1.2
50WTRB	13.6 ± 0.3	29.3 ± 2.7	3 ± 0.1	> 167	> 250	21.3 ± 3.2	34.1 ± 3.4
50WTRBL	6.7 ± 0.2	12.8 ± 0.3	22.8 ± 0.3	> 167	> 250	38.5 ± 1.3	142 ± 6
100WTRB	10.3 ± 1.1	82.8 ± 4.3	4.1 ± 0.2	> 167	> 250	39.6 ± 2.5	63.5 ± 2.7
100WTRBL	2.7 ± 0.1	20.8 ± 1	> 100	> 167	> 250	> 167	> 250
Acarbose			105 ± 4				
Quercetin				32.1 ± 2.4	2.7 ± 0.1	3.9 ± 0.2	
BPII							14.5 ± 1.5

[a] TPC values were expressed as gallic acid equivalent (g GAE)/100 g extracts.
[b] TCC values were expressed as glucose equivalent (g Glc)/100 g extracts.
[c] The IC_{50} value (μg/mL) given in the table is presented as mean ± standard deviation (n=3).

showed the highest DPPH radical scavenging activities, followed by 50 °C and 100 °C water extracts. The activities of 96TRB and 50ETRB were higher than TRB, which indicated that ethanol extraction with ASE is a better extraction procedure for DPPH radical scavengers than BWE.

The IC_{50} values for complement fixation activity of crude extracts ranged from 30 to > 250 μg/mL. The most active fraction was found in TRBL from BWE, while the lowest was found in 100WTRBL. Thus, extraction with boiling water would seem to be a fairly efficient procedure for extracting complement active substances, compared to automated solvent extraction. For the ASE, the original extracts showed higher complement fixation activity compared to LMW fraction, which indicates that some of the activity is present in HMW fractions.

3.2. Extracts from stem bark

The TPC, TCC, IC_{50} values for α-glucosidase, 15-LO and XO inhibition, DPPH radical scavenging and complement fixation activity of extracts from stem bark of *Terminalia macroptera* are summarized in Table 2. The results obtained from stem bark were similar to those for root bark. The 96TSB extract showed the highest TPC and 100WTSB showed the highest TCC. The 96TSB exhibited the lowest IC_{50} values for α-glucosidase (0.6 ± 0.1 μg/mL), 15-LO (33.3 ± 2.4 μg/mL) and XO (43.7 ± 5.1 μg/mL) inhibition and DPPH radical scavenging assay (4.6 ± 0.4 μg/mL) compared to the other extracts. In the complement assay, the 100WTSB showed the highest activity, followed by TSBL. The original extracts exhibited higher values for TPC and TCC than LMW fractions, both from BWE and ASE, as was the case for all the biological assays, the original extracts showing higher activities than their LMW fractions. The extracts from ASE showed higher TPC, TCC and biological activities compared to BWE.

3.3. Extracts from leaves

The characteristics of extracts from leaves of *Terminalia macroptera* are shown in Table 3. The TL extract showed the highest TCC, and the 50ETLL showed the highest TPC and the lowest IC_{50} values for α-glucosidase, 15-LO and XO inhibition and DPPH radical scavenging compared to other extracts. In the complement assay, the 50ETL showed the highest activity, followed by TLL. The original extracts exhibited higher amount of TPC and TCC than LMW fractions (except 50% ethanol–water extracts), both from BWE and ASE, as was the case for all the biological assays, the original extracts showing higher activities than their LMW fractions.

3.4. Brine shrimp toxicity assay

The LC_{50} values against brine shrimp larvae for the tested 27 extracts were much higher than 100 μg/mL. All extracts demonstrated low toxicity against brine shrimp larvae compared to the positive control, podophyllotoxin (80% lethality at 50 μg/mL). Thus, none of the 27 different extracts are toxic against brine shrimp larvae in the test concentrations. These results are in accordance with a previous study on leaf extracts and isolated compounds (Pham et al., 2014).

Table 2
Total phenolic content, total carbohydrate content, α-glucosidase, 15-LO and XO inhibition, DPPH radical scavenging and complement fixation activity of extracts from stem bark of *Terminalia macroptera*.

	TPC GAE ± S.D.	TCC Glc ± S.D.	α-Glucosidase IC_{50} ± S.D.	15-LO IC_{50} ± S.D.	XO IC_{50} ± S.D.	DPPH IC_{50} ± S.D.	Complement fixation IC_{50} ± S.D.
TSB	24.7 ± 1.8	47.0 ± 0.8	1.1 ± 0.1	64.1 ± 3.0	> 250	8.0 ± 0.3	56.9 ± 1.9
TSBL	26 ± 0.7	22.7 ± 3.5	0.9 ± 0.1	56.5 ± 3.2	133 ± 6	9.0 ± 0.4	31.6 ± 3.5
96TSB	31.0 ± 0.7	31.2 ± 2.7	0.6 ± 0.1	33.3 ± 2.4	43.7 ± 5.1	4.6 ± 0.4	> 250
50ETSB	32.1 ± 0.4	63.6 ± 2.6	1.3 ± 0.1	57.6 ± 2.5	167 ± 6	8.3 ± 0.4	44.6 ± 3.2
50ETSBL	9.0 ± 0.1	20.5 ± 3.0	> 100	> 167	> 250	20.5 ± 1.0	> 250
50WTSB	17.3 ± 0.1	43.4 ± 2.9	2.9 ± 0.1	> 167	> 250	39.0 ± 1.1	37.1 ± 4.8
50WTSBL	8.7 ± 0.1	21.8 ± 3.4	12.8 ± 0.1	> 167	> 250	33.3 ± 0.5	45.1 ± 2.8
100WTSB	5.7 ± 0.5	73.7 ± 3.5	10.2 ± 2.2	> 167	> 250	58.5 ± 1.7	30.5 ± 6.4
100WTSBL	3.9 ± 0.1	12.7 ± 2.5	> 100	> 167	> 250	> 167	151 ± 5
Acarbose			104.8 ± 3.9				
Quercetin				32.1 ± 2.4	2.7 ± 0.1	3.9 ± 0.2	
BPII							14.5 ± 1.5

Table 3
Total phenolic content, total carbohydrate content, α-glucosidase, 15-LO, and XO inhibition, DPPH radical scavenging and complement fixation activity of extracts from leaves of *Terminalia macroptera*.

	TPC GAE ± S.D.	TCC Glc ± S.D.	α-Glucosidase IC_{50} ± S.D.	15-LO IC_{50} ± S.D.	XO IC_{50} ± S.D.	DPPH IC_{50} ± S.D.	Complement fixation IC_{50} ± S.D.
TL	15.4 ± 0.1	38.4 ± 5.7	2.5 ± 0.2	51.3 ± 0.9	249 ± 30	17.2 ± 0.6	65.2 ± 4.0
TLL	6.0 ± 0.3	30.8 ± 3.8	18.5 ± 1.5	116 ± 4	> 250	17.7 ± 0.2	45.9 ± 1.6
96TL	19.7 ± 0.4	29.1 ± 4.1	1.7 ± 0.1	73.9 ± 2.7	47.6 ± 5.3	7.5 ± 0.7	> 250
50ETL	18.8 ± 0.5	37.5 ± 1.2	0.7 ± 0.1	54.5 ± 2	86.5 ± 9.0	5.4 ± 0.2	25.2 ± 1.8
50ETLL	27.3 ± 0.6	33.3 ± 3.7	0.6 ± 0.1	36.6 ± 1.2	48.2 ± 4.4	5.3 ± 0.1	52.1 ± 3.0
50WTL	7.1 ± 0.2	24.1 ± 1.7	3.3 ± 0.1	> 167	> 250	48.5 ± 1	66.3 ± 4.1
50WTLL	1.0 ± 0.1	16.1 ± 0.4	> 100	> 167	> 250	> 167	> 250
100WTL	16.6 ± 0.3	36.0 ± 3.1	2.5 ± 0.5	142 ± 34	> 250	20.9 ± 0.7	64.9 ± 7.2
100WTLL	2.4 ± 0.1	17.1 ± 0.7	> 100	> 167	> 250	> 167	66.1 ± 1.4
Acarbose			105 ± 4				
Quercetin				32.1 ± 2.4	2.7 ± 0.1	3.9 ± 0.2	
BPII							14.5 ± 1.5

Table 4
Pearson's correlation coefficients (R) of biological assays (α-glucosidase, 15-LO and XO inhibition, DPPH scavenging, complement fixation), total phenolic content (TPC) and total carbohydrate content (TCC) of extracts from root bark, stem bark and leaves of *Terminalia macroptera*.

	TPC	TCC	α-Glucosidase	15-LO	XO	DPPH	Complement fixation
TPC	1	0.279	0.861**	0.827**	0.677**	0.895**	0.127
TCC		1	0.148	0.136	0.038	0.135	0.365
α-Glucosidase			1	0.807**	0.786**	0.908**	0.063
15-LO				1	0.707**	0.896**	0.069
XO					1	0.758**	−0.222
DPPH						1	0.095
Complement fixation							1

Table 5
Characteristic values, accumulated contribution and feature vector of principal component analysis.

Principal component:	1	2		Variable
Characteristic value	3.436	1.086		
Accumulated contribution (%)	68.72	90.44		
Feature vector	0.947	0.060	X_1	α-glucosidase
	0.922	0.092	X_2	15-LO
	0.872	−0.292	X_3	XO
	0.964	0.108	X_4	DPPH
	0.008	0.989	X_5	Complement fixation

3.5. Correlation analysis

A direct correlation analysis among the five biological assays, as well as among TPC, TCC and the biological activities of 27 different extracts, was carried out by Pearson's correlation analysis. For the biological assays, lower IC_{50} values mean higher activities. In this case, all IC_{50} values were converted into $1/IC_{50}$ values. For inactive samples (IC_{50} values above the highest measured concentration), these values were set to zero for correlation analysis. The results of correlation analysis are shown in Table 4.

As shown in Table 4, α-glucosidase, 15-LO and XO inhibition and DPPH scavenging activity were highly correlated with TPC. Also, there were significant correlations between the different enzyme inhibitory activities and between these and the DPPH scavenging activity. Wangensteen et al. (2004) also found that TPC was significantly correlated to DPPH scavenging activity and 15-LO inhibition activity. In our study, the results of the correlation analyses indicate that the α-glucosidase, 15-LO and XO inhibition activities and the DPPH scavenging activity were expressed mainly by the phenolic compounds. These findings are reasonable, since isolated phenolic compounds from leaves of *Terminalia macroptera* have shown potent 15-LO, XO and α-glucosidase inhibition and DPPH scavenging activity (Pham et al., 2011b, 2014). Thus, a screening of phenolic content in *Terminalia macroptera* extracts will probably indicate the presence of compounds with enzyme inhibitory and antioxidant activities. No significant correlation was found among complement fixation activities and TPC and TCC, as well as other biological activities. The TCC exhibited no correlation with TPC and biological activities either. Although the slight correlation between TCC and complement fixation is not statistically significant, some purified polysaccharide fractions obtained from ASE crude extracts of *Terminalia macroptera* showed strong complement fixation activity (Zou et al., in press). These results indicated that the complement fixation activities present in crude extracts are not only due to polysaccharides, but might arise from some LMW compounds as well (Pieters et al., 1999).

The root bark and leaf extracts have higher TPC and TCC than their LMW fraction, as well as higher biological activities in most of the assays (Tables 1 and 3). For the stem bark, TPC values and biological activities are fairly similar for the total extract and the LMW fraction (Table 2). This is reflected in the high correlation between TPC and enzyme inhibitory and radical scavenging activities (Table 4). The TCC in HMW fractions is most probably due to polysaccharides; thus one would expect that the original fractions have higher complement fixation activities than their LMW fractions. Various plant-derived polysaccharides have shown high complement fixation activity (Paulsen and Barsett, 2005), and some purified polysaccharide fractions with strong complement fixation activity were obtained from ASE crude extracts of *Terminalia macroptera* (Zou et al., in press).

Table 6
Comprehensive principal value and rank of extracts from *Terminalia macroptera*.

	F_1	F_2	F
96TRB	7.46	−1.52	4.79
96TSB	7.06	−1.45	4.53
50ETLL	6.22	0.21	4.32
50ETL	4.14	2.14	3.31
50ETRB	4.32	0.11	2.99
TSBL	2.23	1.42	1.84
TRB	2.07	0.71	1.58
96TL	2.56	−1.78	1.37
50ETSB	1.68	0.65	1.30
TSB	1.23	0.41	0.94
TL	0.31	−0.01	0.21
TRBL	−0.96	1.58	−0.31
50ETRBL	−0.81	0.13	−0.53
100WTL	−1.40	−0.02	−0.96
TLL	−1.63	0.53	−1.01
50WTRB	−2.18	1.11	−1.26
50WTSB	−2.46	0.87	−1.50
100WTSB	−2.93	1.34	−1.72
50WTL	−2.60	−0.17	−1.82
100WTRB	−2.62	−0.11	−1.82
50WTSBL	−2.79	0.44	−1.82
50ETSBL	−2.67	−1.46	−2.15
50WTRBL	−2.91	−0.88	−2.19
100WTLL	−3.32	−0.22	−2.33
100WTSBL	−3.33	−0.96	−2.50
100WTRBL	−3.33	−1.53	−2.62
50WTLL	−3.33	−1.53	−2.62

3.6. Principal component analysis

In our study, five different biological assays were investigated. As shown in Table 4, there are complex relations among the five biological assays. The principal component analysis (PCA) is known to reduce dimensionality of a data set while simultaneously retaining the information present in the original data (Huang et al., 2013). In this case, PCA was applied to process the IC_{50} values for comparative biological activities profiling of different extracts from root bark, stem bark and leaves from *Terminalia macroptera*.

The results of PCA of 5 biological assays are shown in Table 5. The characteristic values of the first two principal components were more than 1; the accumulated contribution of these two

components was 90.44%. This means these two new components including 90.44% information of the five original components, which indicates that these two principal components can be substituted for the primary 5 components.

There are four factors in the first new component, α-glucosidase, 15-LO and XO inhibition and DPPH scavenging. This makes sense, since significant correlations were found among four of them (Table 4). The complement fixation activity was the major factor in the second component which is quite different from enzyme inhibition and antioxidant activity. According to the feature vector, the linear equations of the principal components are:

$$F_1 = 0.947ZX_1 + 0.922ZX_2 + 0.872ZX_3 + 0.964ZX_4 + 0.008ZX_5$$

$$F_2 = 0.060ZX_1 + 0.092ZX_2 - 0.292ZX_3 + 0.108ZX_4 + 0.989ZX_5$$

where ZX_j is the standardization value of original variable X_j.

The comprehensive evaluation equation was obtained after enter ZX_j and accumulated contribution into the linear model

$$F = 0.6872F_1 + 0.2172F_2$$

Table 6 shows the comprehensive principal values through the equation.

According to the comprehensive principal value, 27 different extracts could be divided into two groups, the comprehensive principal values of first group were positive, and the second group was negative. The higher principal value indicates higher total activities. Table 6 shows that 96TRB has the highest principal value among 27 different extracts, followed by extracts 96TSB and 50ETLL, while the lowest principal value was found in 100TRBL and 50TLL. Based on these five biological assays, we can use different extraction procedures to get the wanted activities using root bark, stem bark and leaves of *Terminalia macroptera*.

4. Conclusions

In this study, significant correlations were found among enzyme inhibition (α-glucosidase, 15-LO, XO), DPPH scavenging activity and TPC; thus a screening of phenolic content in *Terminalia macroptera* extracts will probably indicate the presence of compounds with enzyme inhibitory and antioxidant activities. Several of the extracts from root bark, stem bark and leaves of *Terminalia macroptera* obtained by BWE and ASE showed potent enzyme inhibition activities, radical-scavenging properties and complement fixation activities. The present study demonstrated that 96TRB, 96TSB and 50ETLL, obtained by ASE from *Terminalia macroptera*, possessed the strongest comprehensive biological activities as compared to other extracts. Of the boiling water extracts, TRB (from root bark) had the highest value for both principal components. Using leaves for traditional medicines will reduce serious damage to the tree compared to the use of root and stem bark. Of the boiling water extracts, the root bark gave the highest total activity, followed by the stem bark, and the leaves gave somewhat less active extracts. α-Glucosidase inhibition was, however, strong in all these extracts. For sustainability, LMW fraction from 50% ethanol–water extracts of leaves obtained by ASE seems preferable. It is, however, noteworthy that some of the hot water extracts have activity in several of the assays. This might be seen as a rationale for the traditional medicinal use of the plant (Pham et al., 2011a). Some of the active constituents of the leaves have been identified (Pham et al., 2011b, 2014). In future work, it would be of interest to analyze further active compounds present in the crude extracts.

Acknowledgments

The project is part of the FP7 EU project MUTHI, project number 266005. The first author is also grateful for receiving funding from the China Scholarship Council. The authors are indebted to Helle Wangensteen, Department of Pharmaceutical Chemistry, University of Oslo, for the great help of brine shrimp toxicity assay.

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